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ORIGINAL ARTICLE

Ambulatory blood pressure and left ventricular structure and function in relation to the G-protein β_3-subunit polymorphism *C825T* in White Europeans

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The *825T* allele of the G-protein β_3-subunit is associated with increased intracellular signalling. Its association with hypertension is inconsistent. We, therefore, studied the C825T polymorphism in relation to ambulatory blood pressure as well as left ventricular structure and function in two European populations. We genotyped 248 parents and 318 offspring, enrolled in the European Project on Genes in Hypertension in Cracow, Poland ($n = 286$) and in Novosibirsk, Russian Federation ($n = 280$). The 24-h ambulatory blood pressure was recorded using oscillometric SpaceLabs 90207 monitors. Within each centre, a single observer performed two-dimensionally guided M-mode echocardiography and Doppler sonography to measure left ventricular structure (American Society of Echocardiography conventions) and diastolic function: early (E) and late (A) peak diastolic inflow velocities. We used analysis of covariance and generalized estimating equations to allow for covariables and nonindependence among related subjects. Genotype frequencies were similar ($P = 0.25$) in Cracow and Novosibirsk and amounted to 44.7% for *CC*, 47.2% for *CT,* and 8.1% for *TT*. Among parents (mean age: 51.3 years)—but not among offspring (mean age 25.1 years)—24-h, daytime and night time systolic blood pressures were 5–6 mmHg higher in *TT* homozygotes than in *C* allele carriers. In *TT* homozygous parents (−8.2 cm/sec, $P = 0.004$) as well as in *TT* homozygous offspring (−7.5 cm/sec, $P = 0.02$), the E-wave was significantly reduced, which in offspring also resulted in a lower E/A ratio (–0.25, $P = 0.002$). Neither in parents nor in offspring, left ventricular mass index was associated with the *C825T* polymorphism. In conclusion, in *TT* homozygotes of both generations, early left ventricular relaxation was reduced. In *TT* homozygous parents, the latter observation might be because of the higher systolic pressure associated with the *TT* genotype.
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Introduction

Guanine nucleotide regulatory proteins (G-proteins) are part of many intracellular signalling cascades. [1] Their activation is the principal mechanism through which stimulated heptahelical receptors generate changes in intracellular function.[1] Siffert *et al*[2] identified a common *C825T* polymorphism in exon 10 of the β_3-subunit of the heterotrimeric G-protein. The *825T* allele has been associated with a splice variant, which shortens the protein by 41 amino acids. This mutation leads to a gain in function[2] and entails stimulation of the ubiquitously expressed Na^+/H^+ exchanger.[3,4] de la Sierra *et al*[5] observed that in patients with essential hypertension, left ventricular hypertrophy was associated with increased Na^+/H^+ exchanger activity. In addition,

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several studies[6–8] reported that disorders such as obesity and hypertension, which may cause left ventricular hypertrophy, are associated with the G-protein β_3-subunit (GNB3) gene *C825T* polymorphism. We, therefore, investigated the possible impact of this genetic polymorphism on ambulatory blood pressure as well as left ventricular structure and function in White Europeans.

Methods

General outline of the study

The primary goal of the European Project on Genes in Hypertension (EPOGH) Study was to investigate the complex relation between blood pressure analysed as a continuous or binary phenotype and various candidate genes.[9] In addition to blood pressure, several other intermediate or associated phenotypes, such as left ventricular mass were measured. The epidemiological methods used in EPOGH have been previously validated.[9–11] The project was conducted according to the principles outlined in the Helsinki declaration for investigations in human subjects.[12] Each local Institutional Review Board approved the study. Participants gave informed written consent.

Study population

Nuclear families were recruited in seven countries (Belgium, Bulgaria, the Czech Republic, Italy, Poland, Romania, and the Russian Federation).[9] Families had to include at least one parent and two siblings. The age range for participation was 18–60 years. For the present analysis, the study population was limited to 631 subjects recruited by the two centres that, at the time of writing of this manuscript, had participated in the optional substudy on echocardiography: Cracow (Poland, Centre 1) and Novosibirsk (the Russian Federation, Centre 2). Subjects were not considered for analysis (1) if they had declined the invitation for the echocardiographic examination ($n=49$), (2) if the echocardiogram was of bad quality ($n=7$), (3) if the subjects had impairment of left ventricular systolic function ($n=3$), or a valvular disorder ($n=1$), or (4) if their DNA did not amplify ($n=5$).

Trained observers measured blood pressure five times consecutively during each of two home visits after the subjects had rested for 5 min in the sitting position. The guidelines of the British Society of Hypertension were applied.[13] Each subject's conventional blood pressure was the mean of five consecutive readings obtained at the second contact with the subject.[9] Hypertension was diagnosed if the average of the five readings was at least 140 mmHg systolic or 90 mmHg diastolic, or when the subjects were on antihypertensive drug treatment.

Validated oscillometric SpaceLabs 90207 monitors (Redmond, WA, USA) fitted with the same cuff size as for conventional measurements were programmed to obtain readings with an interval of 15 min from 08:00 to 22:00 h and every 30 min from 22:00 to 08:00 h. In keeping with current recommendations,[14] daytime was defined as the interval from 10:00 to 22:00 h and night time from midnight to 06:00 h. These definitions eliminate the transition periods in the morning and evening, during which rapid blood pressure changes occur in most subjects.

Via a standardized interview, information was collected on each participant's personal and familial medical history, smoking and drinking habits, physical activity, and use of medications. Body surface area (BSA; m^2) was calculated as body weight $(kg)^{0.425} \times$ body height $(cm)^{0.725} \times 71.84$.[15]

Echocardiographic methods

In each centre, one experienced observer (AO and AR) performed all echocardiograms, using a commercially available ultrasonograph equipped with a 3.5-MHz. transducer with the subjects in left decubitus position. M-mode echocardiograms of the left ventricle were obtained at end-expiration from the parasternal long-axis view under control of the two-dimensional image. The ultrasound beam was positioned just below the mitral valve at the level of the posterior chordae tendineae. Left ventricular internal dimension (LVID) and interventricular septal (IVST) and posterior wall thickness (PWT) were measured at end-diastole according to the recommendations of the American Society of Echocardiography, using the leading edge-to-leading edge convention.[16,17] For statistical analysis, the measurements of three cardiac cycles were averaged. Studies were recorded on videotapes. End-diastolic left ventricular dimensions were used to calculate left ventricular mass by an anatomically validated formula.[18] The relative wall thickness was calculated as (IVST+PWT)/LVID. The intraobserver intersession reproducibility coefficient for left ventricular mass was 2.5% for Centre 1 and 2.0% for Centre 2. Left ventricular mass index was calculated as left ventricular mass divided by BSA.[18] As a measure of systolic function, dimensional fractional shortening was computed.[18] For evaluation of diastolic function, mitral inflow velocities were recorded with pulsed-wave Doppler sonography. Pulsed Doppler spectral recordings were obtained from the apical four-chamber view with a sample volume positioned at the tips of the mitral leaflets. Three consecutive cardiac cycles were averaged to measure peak velocities reached in early diastole (E-wave) and after atrial contraction (A-wave),[19] and to calculate the E/A ratio.

Determination of the genotype

Genomic DNA was extracted from white blood cells. From the published DNA sequence of the GNB3,[20] we amplified a 268 base pair fragment incorporating

the polymorphic site with the use of two primers as described by Siffert *et al*.[21] We previously published the technical procedures, which we implemented for DNA amplification and determination of the genotypes by allele-specific hybridization.[22]

Statistical analysis

We used the SAS software package, version 6.12 (SAS Institute, Cary, NC, USA) for database management and statistical analysis. Comparisons of means and proportions involved the standard normal *z*-test and the χ^2-statistic, respectively. Regression slopes were compared using a multiple regression approach as described by Kleinbaum *et al*.[23] We first searched for possible confounders using stepwise multiple regression with the *P*-value for covariables to enter and stay in the model set at 0.15.

Continuous traits adjusted for covariables were first analysed by analysis of covariance. Since genetic heterogeneity among pathophysiologically related phenotypes consistently suggested a recessive pattern, we subsequently only compared *TT* homozygotes with *C* allele carriers, using the least-square mean option of the PROC GLM procedure. Since family members are more likely to share identical alleles than randomly selected subjects and to allow for the nonindependence of the cardiovascular phenotypes within families, we repeated our analyses using generalized estimating equations (GEE) as implemented in the PROC GENMOD procedure of the SAS package.[24] In GEE analyses, we adjusted for confounders, we treated families as clusters and family members as repeated observations within clusters, and we defined the working correlation matrix as unstructured.

Results

Characteristics of the study participants

Our study population ($n=566$) included 248 parents and 318 offspring. Their clinical, demographic and echocardiographic characteristics are reported in Table 1. Mean age ($\pm$ s.d.) was 51.3 $\pm$ 5.3 years in parents and 25.1 $\pm$ 5.0 years in offspring. Body mass index (28.7 $\pm$ 4.9 *vs* 22.7 $\pm$ 3.2 kg/m^2), 24-h blood pressure (124.5 $\pm$ 13.3/77.1 $\pm$ 8.7 *vs* 116.0 $\pm$ 8.9/68.4 $\pm$ 6.4 mmHg), and left ventricular mass index (106.9 $\pm$ 25.5 *vs* 85.5 $\pm$ 17.4 g/m^2) were higher in parents than offspring ($P<0.001$ for all). The prevalence of hypertension based on conventional blood pressure measurement was higher in parents than offspring (54.4 *vs* 10.4%, $P=0.001$). Peak early diastolic inflow velocity was lower in parents than offspring (66.2 $\pm$ 17.3 *vs* 85.1 $\pm$ 15.3 cm/s; $P<0.001$), whereas the opposite was observed for peak late diastolic wave velocity (64.5 $\pm$ 14.1 *vs* 51.1 $\pm$ 11.4 cm/s; $P<0.001$). As a consequence, the E/A ratio was lower in parents than offspring (1.06 $\pm$ 0.32 *vs* 1.72 $\pm$ 0.41, $P<0.001$). A total of 56 parents (22.6%) were current smokers and 73 (29.4%) reported intake of alcohol ($\geqslant$5 g of ethanol per day). Among offspring, these numbers were 99 (31.1%) and 115 (36.2%), respectively. Gender differences showed similar trends in both generations and are reported in Table 1.

Genotype and allele frequencies

The genotype frequencies of the *C825T* polymorphism did not significantly deviate from Hardy–Weinberg equilibrium in Novosibirsk ($P=0.81$) or Cracow ($P=0.06$). Genotype and allele frequencies

Table 1 General characteristics of offspring and parent generations

	Sons N=*151*	*Daughters* N=*167*	*Fathers* N=*100*	*Mothers* N=*148*
Clinical characteristics				
Age (years)	24.5 (4.7)	25.6 (5.2)	52.0 (5.1)	50.9 (5.5)
Body mass index (kg/m^2)	23.1 (3.0)	22.3 (3.4)*	27.3 (4.1)	29.6 (5.2)***
Systolic pressure (mmHg)[a]	124.5 (12.6)	111.5 (10.3)***	134.3 (18.0)	136.4 (19.3)
Diastolic pressure (mmHg)[a]	77.1 (9.8)	71.4 (7.9) ***	86.8 (11.8)	86.7 (10.0)
Hypertension (*n*)	26 (17.2%)	7 (4.2%)***	51 (51.0%)	85 (57.0%)
Hypertension treated (*n*)	10 (6.6%)	3 (1.8%)	21 (21.0%)	58 (39.2%) **
Echocardiographic measurements				
Left ventricular mass (g)	178.5 (36.8)	130.5 (28.2)***	227.1 (58.6)	181.9 (48.5)***
Left ventricular mass index (g/m^2)	93.6 (16.8)	78.2 (14.4)***	115.5 (25.5)	101.1 (23.9)***
Left ventricular mass/height $^{2.7}$ (g/m$^{2.7}$)	37.7 (7.6)	33.8 (7.6)***	51.7 (13.8)	50.9 (13.9)
Left ventricular fractional shortening (%)	38.4 (4.9)	39.6 (4.0)***	38.0 (5.7)	41.1 (5.0)***
E-wave (cm/sec)	84.5 (16.1)	85.5 (14.7)	60.7 (15.1)	69.9 (17.8)***
A-wave (cm/sec)	50.2 (11.9)	51.8 (10.9)	62.3 (14.0)	65.9 (14.1)*
E/A ratio	1.74 (0.43)	1.70 (0.37)	1.00 (0.29)	1.10 (0.33)*
Heart rate (bpm)[b]	70.8 (11.4)	72.4 (10.0)	67.9 (12.1)	70.4 (9.8)

[a]Average of five readings at the second contact with the subject.
[b]Heart rate during echocardiography.
Values are arithmetic means (s.d.), or number of subjects (%). Significance of the difference between men and women within each generation: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

also did not differ according to the centre (Table 2), or the presence *vs* the absence of hypertension ($P = 0.54$).

Association analysis

Blood pressure on ambulatory measurement did not differ across genotypes in offspring ($P \geqslant 0.16$;. Table 3). However, in parents (Table 4), the ANOVA *P*-values for systolic pressure on 24-h, daytime and night time ambulatory measurements were smaller than 0.15. Since trends in the systolic measurements consistently suggested a recessive effect of the *T* allele, we subsequently compared *TT* homozygotes with *C* allele carriers. *TT* homozygous parents had higher 24-h, daytime and night time systolic pressures (Table 4 and Figure 1; $P \leq 0.04$). Left ventricular mass index, left ventricular mass/height$^{2.7}$, fractional shortening and heart rate measured during the echocardiographic examination did not differ across genotypes in offspring ($P \geqslant 0.41$; Table 3) or parents ($P \geqslant 0.32$; Table 4). However, in both generations, there was a significant association between peak early diastolic inflow velocity and the GNB3 *C825T* polymorphism, which in offspring also resulted in significant genetic difference in the E/A ratio. Indeed, in both generations, peak early diastolic inflow velocity was consistently lower in *TT* homozygotes than in *C* allele carriers (Tables 3 and 4; Figure 1). Further analyses of peak early diastolic inflow velocity and the E/A ratio did not show a significant gender-by-genotype interaction in either generation ($P \geqslant 0.27$) or a significant generation-by-genotype interaction in either sex ($P \geqslant 0.10$; Figure 2). Overall, for two generations and both sexes combined, *TT* homozygotes compared to *C* allele carriers had significantly lower peak early diastolic inflow velocity (69.7 ± 13.4 *vs* 77.5 ± 13.1 cm/sec) and E/A ratio (1.29 ± 0.33 *vs* 1.45 ± 0.32; Figure 2).

We repeated our analyses with a similar set of covariables as above using GEE instead of analysis of covariance to allow for the nonindependence of the phenotypes within families. The GEE analyses confirmed ANOVA results.

In all subjects combined, the E/A ratio decreased with increasing systolic blood pressure (–0.0023 per mmHg; $P = 0.02$). Furthermore, neither in offspring

Table 2 Genotype and allele frequencies by centre

	Genotypes			*Alleles*	
	CC	CT	TT	C	T
Cracow	124 (43.4%)	143 (50.0%)	19 (6.6%)	391 (68.4%)	181 (31.6%)
Novosibirsk	129 (46.1%)	124 (44.3%)	27 (9.6%)	382 (68.2%)	178 (31.8%)
Total	253 (44.7%)	267 (47.2%)	46 (8.1%)	773 (68.3%)	359 (31.7%)

P-values for between-centre difference in genotype and allele frequencies were 0.25 and >0.99, respectively.

Table 3 Phenotypes by G-protein β_3-subunit genotypes in offspring

	Genotypes statistics			P_{ANOVA}	P *(*TT *vs* CC+CT*)*
	CC n=*144*	*CT* n=*153*	*TT* n=*21*		
Clinical measurements					
24-h systolic pressure (mmHg)	116.5 (0.6)	115.7 (0.6)	115.0 (1.6)	0.55	—
24-h diastolic pressure (mmHg)	68.8 (0.5)	68.2 (0.5)	67.6 (1.3)	0.58	—
Daytime systolic pressure (mmHg)	123.4 (0.7)	123.0 (0.7)	122.4 (1.8)	0.85	—
Daytime diastolic pressure (mmHg)	75.3 (0.6)	74.9 (0.6)	74.1 (1.5)	0.75	—
Night time systolic pressure (mmHg)	105.8 (0.7)	104.2 (0.7)	103.3 (1.8)	0.16	—
Night time diastolic pressure (mmHg)	58.6 (0.5)	57.7 (0.5)	57.3 (1.4)	0.38	—
Echocardiographic data					
Left ventricular mass index (g/m^2)	85.1 (1.3)	85.7 (1.2)	87.3 (3.4)	0.81	—
E-wave (cm/sec)	85.9 (1.1)	85.2 (1.1)	78.2 (2.9)	0.05	0.02
A-wave (cm/sec)	50.6 (0.8)	51.2 (0.8)	53.1 (2.1)	0.55	—
E/A ratio	1.75 (0.03)	1.73 (0.03)	1.49 (0.08)	0.008	0.002

Values are means (s.e.) adjusted for centre, sex and age. For blood pressure additional covariables accounted for in the analysis were body mass index and use of antihypertensive medication. For left ventricular mass index, additional covariables were systolic blood pressure and use of antihypertensive medication, and for left ventricular Doppler measurements also heart rate.
TT homozygotes and *C* allele carriers were compared, if the ANOVA *P*-value was $\leqslant 0.15$.

Table 4 Phenotypes by G-protein β_3-subunit genotypes in parents

	Genotypes statistics			P_{ANOVA}	P *(TT vs* CC+CT*)*
	CC n=*109*	CT n=*114*	TT n=*25*		
Clinical measurements					
24-h systolic pressure (mmHg)	124.0 (1.2)	124.0 (1.1)	129.5 (2.4)	0.10	0.03
24-h diastolic pressure (mmHg)	76.8 (0.8)	76.9 (0.8)	79.1 (1.7)	0.43	—
Daytime systolic pressure (mmHg)	129.6 (1.2)	129.5 (1.2)	135.0 (2.5)	0.13	0.04
Daytime diastolic pressure (mmHg)	81.9 (0.8)	81.9 (0.8)	83.8 (1.8)	0.59	—
Night time systolic pressure (mmHg)	113.1 (1.2)	114.3 (1.2)	119.6 (2.6)	0.08	0.03
Night time diastolic pressure (mmHg)	67.8 (0.9)	68.6 (0.8)	70.8 (1.8)	0.29	—
Echocardiographic data					
Left ventricular mass index (g/m^2)	107.9 (2.1)	105.0 (2.0)	111.6 (4.3)	0.32	—
E-wave (cm/sec)	67.3 (1.3)	66.8 (1.3)	58.9 (2.7)	0.02	0.004
A-wave (cm/sec)	64.3 (1.2)	64.6 (1.1)	64.1 (2.4)	0.96	—
E/A ratio	1.08 (0.03)	1.06 (0.03)	0.99 (0.05)	0.25	—

For explanation, see Table 3.

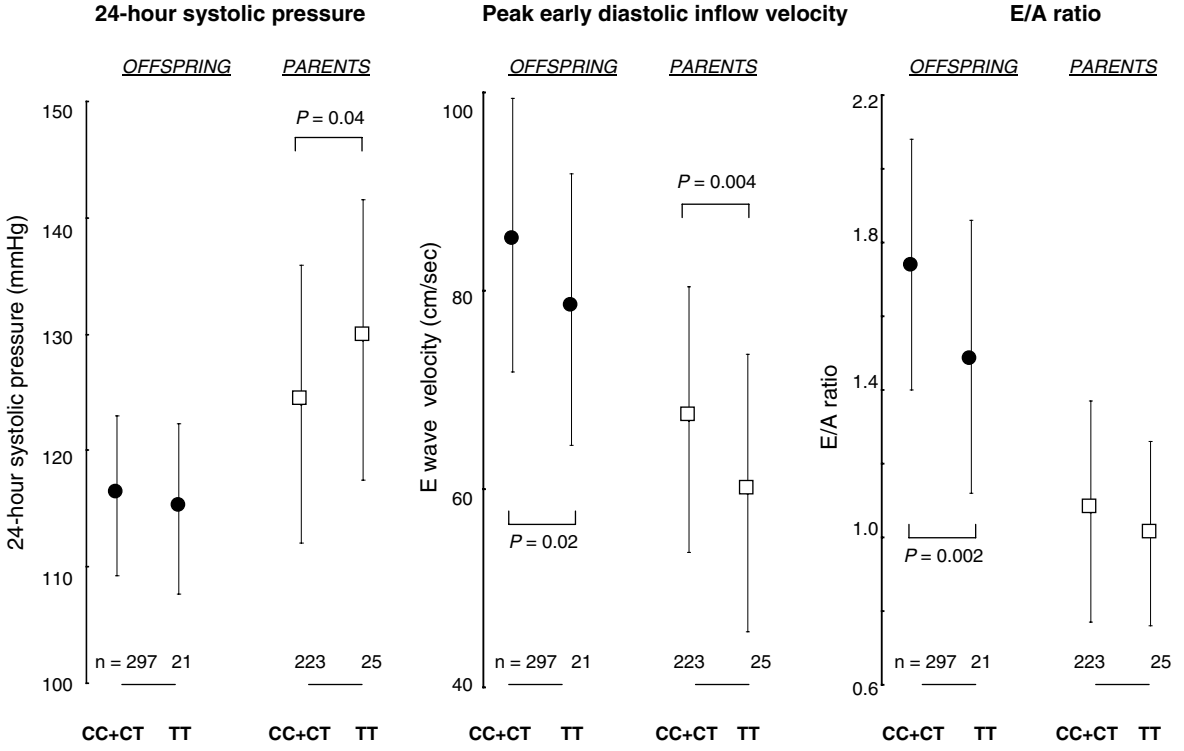

Figure 1 Ambulatory 24-h systolic pressure and left ventricular diastolic function by generation and GNB3 genotype. The analyses were adjusted for centre, sex, age, body mass index, and use of antihypertensive medications. Left ventricular diastolic function was also adjusted for heart rate.

($P=0.62$) nor in parents ($P=0.30$), the slope of the E/A ratio on systolic blood pressure differed between *TT* homozygotes and *C* allele carriers.

Discussion

We studied phenotypic variation associated with the GNB3 *C825T* polymorphism in two European populations. Our main finding was that left ventricular relaxation, as reflected by peak early diastolic inflow velocity, was reduced in *TT* homozygotes of both the parent and the offspring generation. Moreover, in *TT* homozygous parents, systolic blood pressure measured by ambulatory monitoring was approximately 5 mmHg higher than in *C* allele carriers. Only one small study in 34 white patients with essential hypertension found a

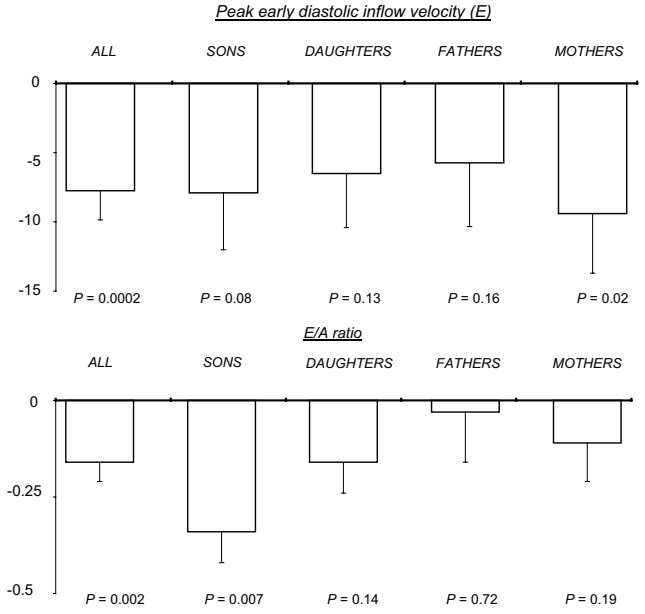

Figure 2 Difference in left ventricular diastolic function associated with *TT* homozygosity of the GNB3 gene by sex and generation. The reference lines represent the mean in *C* allele carriers. For adjustments, see Figure 1.

reduced transmitral blood flow in relation to the GNB3 *825T* allele.[25] Two other reports involving hypertensive patients with and without left ventricular hypertrophy demonstrated a positive association between left ventricular mass and the *825T* allele.[26,27] In contrast, a large population-based study did not reveal any association between cardiac structure and GNB3 *T* allele.[28]

Association never proves causation. Nevertheless, several mechanisms may explain why early left ventricular relaxation may be impaired in the presence of the 825T allele. First, in *TT* homozygous parents we observed a 5 mmHg higher systolic pressure throughout the whole day. Impaired left ventricular diastolic filling is an early marker of hypertensive heart disease and precedes the structural adaptation of the myocardium to high blood pressure.[29] However, this mechanism is unlikely to account fully for the reduced early diastolic filling in *TT* homozygous offspring, because among siblings the ambulatory blood pressure was similar across genotypes. Age is also an important determinant of left ventricular structure and function. However, the relative decrease in peak early diastolic inflow velocity in *TT* homozygotes compared to *C* allele carriers was similar in offspring and parents, averaging approximately 10%. Furthermore, neither in offspring nor in parents, the slope of the E/A ratio on systolic blood pressure differed between *TT* homozygotes and *C* allele carriers.

In addition to blood pressure, age and their interaction, molecular mechanisms may also explain our findings. In cardiomyocytes, the extrusion and sequestration of free calcium during diastole is dependent on cyclic AMP, is modulated by β-receptors, and is influenced by several ion transport mechanisms including the Na^+/H^+ exchanger.[30–36] Cardiac β-receptors couple to a G-protein-stimulated adenylcyclase, which in turn stimulates cyclic AMP-dependent regulatory mechanisms.[30,31,33,37] Recent studies in rodent cardiomyocytes revealed that β_2-receptors couple to G-proteins, which lead to inhibition of cyclic AMP formation downstream.[38,39] In the ischaemic myocardium, the Na^+/H^+ exchanger, which itself is under the influence of the C825T polymorphism,[3,4] exerts an important modulatory action on cardiac excitation–contraction coupling.[36]

Published studies on the possible influence of the GNB3 *C825T* polymorphism reported inconsistent results. The *825T* allele was associated with increased blood pressure[40–42] or increased risk of hypertension[22,43,44] in Germans,[22,39,42,44] Australian White hypertensive patients[41] and Caribbean or West African Blacks,[43] but not in French,[22] Irish,[22] Japanese[45,46] or African Americans.[47] These discordant findings suggest that ethnic or genetic background,[8] lifestyle or various environmental factors may modulate the potential effect of the *C825T* allele on blood pressure. The frequency of the *T* allele was 0.32 in our Caucasian populations, but it is approximately 0.50 in Japanese[45,46] or native Americans[48,49] and 0.75[42,46] in black populations. One possible mechanism for the higher systolic blood pressure might be chronic sodium retention and expansion of the circulating volume as a consequence of the higher activity of the Na^+/H^+ exchanger.[2,4] Several observations support this hypothesis. In one population-based study, plasma renin activity was decreased in the presence of the *T* allele.[40] Turner *et al*[49] found in black and non-Hispanic white populations with essential hypertension that systolic and diastolic blood pressure declined 5–6 mmHg more in response to diuretic treatment in *TT* than *CC* homozygotes. Zeltner *et al*[3] found in young men with normotension or mild incipient essential hypertension (mean age 26 years) that carriers of the *825T* allele had faster tubular sodium reabsorption than *CC* homozygotes. However, in the present study, end-diastolic left ventricular diameter did not increase with the number of *T* alleles as one might expect in the case of a volume-dependent rise in systolic pressure. Discordance among the published reports on hypertension in relation to the *C825T* polymorphism[22,23,40–47] might also be because of the large variability in the blood pressure phenotype. Indeed, in most studies it was only measured on a single occasion by conventional sphygmomanometry.

In conclusion, in *TT* homozygotes compared to *C* allele carriers, left ventricular relaxation, as reflected by the early peak diastolic inflow velocity, was reduced. In *TT* homozygous parents, this observation may be because of the higher systolic pressure associated with this genotype.

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